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Assessment of Immunoglobulins In The Serum Of Children With Shigellosis

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Abstract

During a period of nine months from January 2006 to September 2006. Sixty –two cases of shigellosis were collected at Nassiria Pediatric hospital (Age ranged from one day to five years). A total of 317 cases suffering form bloody diarrhea , out of 317 stool samples , 62 bacterial isolates were obtained represent 19.5% from all cases of diarrhea ,The isolates identified were, S*higella dysenteriae* , *Shigella sonnei* , *Shigella boydii* , and *Shigella flexneri* .The percentage of each are , 22(35.4%) , 19(30.6%) , 13(20.9%) , 8(12.9%) respectively . Shigellosis was predominant in those below one year of age (32.2%) .The concentration of total protein level in the serum of children with shigellosis was normal in all age groups , while albumin was low , alpha 1 – globulin concentration was high , alpha 2 –globulin increased slightly with advancing age , beta – globulin concentration in serum of children was normal , gamma globulin concentration was slightly elevated in most age groups . Forty – seven children (75.8%) are found to have high level of lmmunoglobulins concentration while fifteen children (24.2%) have low levels of lmmunoglobulin concentration .They are classified as follows : 11.2% have IgA deficiency , 5.1% have IgM deficiency and 4.8% have IgG deficiency.These results revealed that Ig deficiency is associated with shigellosis .

Introduction:

Shigellosis (severe bloody diarrhea) is an important cause of diarrheal disease in developing countries [1],and in travelers to tropical countries[2,3]. It becoming one of the leading causes of diarrhea morbidity and mortality in children under five years of age.[4]

Four species of *Shigella* are pathogenic for man. *Shigella sonnei* and *Shigella boydii* usually causes relatively mild illness in which diarrhea may be watery or bloody [5]. *Shigella flexneri* is the chief cause of endemic shigellosis in developing countries, and *Shigella dysenteriae* type 1 causes both epidemic and endemic shigellosis. Although S*higella dysenteriae* type 1 is the *Shigella* species associated with the most severe disease and the highest case fatality rates, the majority of deaths from shigellosis result, from endemic disease, especially that caused by *Shigella flexneri* [6]. Shigellosis is also more severe in children with pre-existing malnutrition, causing their nutritional status to worsen rapidly [4 , 7, 8]. Shigellosis occur in children under five years old.[4]

Among children, the risk of death from shigellosis is greatest in infants and those who are severely malnourished [9]. High case fatality rates (5% and above) may be associated with any of the four species of Shigella (S*higella dysenteriae* type 1, S*higella. Flexneri*, S*higella. Boydii* and S*higella sonnei*) [10]. The virulence factors of the lipopolysaccharide of *Shigella* species bacteria include the endotoxic activities of the lipid A component of the molecule and the ability of the polysaccharide chain–the core and the O-antigenic polysaccharide–to provide the bacterium with resistance to host defense mechanisms such as opsonization, phagocytosis, and intracellular killing [11]. *Shigella* also invades epithelial cells through pathogen-directed endocytosis [12]. Invasion of enterocytes and bacterial cell-to-cell spread enhance tissue damage [13 , 14].

Level of serum protein components, albumin ,alpha1 globulin ,alph2 globulin.beta globulin and gamma globulin were used to diagnosis some diseases by using serum protein electrophoresis .This pattern can provide clues to diagnose some diseases and done to evaluate hypogammaglobulinemia a condition charactrierized by low level of gamma globulin antibodies such as IgG which is predominant during bacterial infection and the IgA subclass distribution of serum antibodies against different common bacterial ,viral and food antigens.[15 , 16 , 17].The aim of this study to determine the frequency of Shigellosis diseases among children and the effect of this infection on the serum proteins and immunoglobulins levels in the serum of children with shigellosis disease .

Materials and Methods:

Patients:

During the period from January 2006 to September 2006 ,Sixty-two stool and blood samples were obtained from pediatric patients (aged from one day to five years old) whom suffering from bloody diarrhea and shigellosis were included in this study .they were admitted to pediatric hospital in Al-Nassiria city . Information obtained at the time of admission included ,age ,sex ,antibiotics intake before admission.,.

Control group:

This group consisted of ten (10) healthy children without any disease

Microbiological methods:

To isolate *Shigella* species, stool samples were inoculated onto MacConkey agar and Salmonella–Shigella agar and the resulting colonies which exhibited characteristics of *Shigella* species were identified by conventional biochemical methods [18]. The macroscopic examination also were done to determine presence of blood and mucus .

Serum protein electrophoresis:

Serum were used to determined the fractionations of the serum proteins by cellulose acetate electrophoresis (Beckaman company ,CDS-200) [19] .

Single Radial Immunodiffusion :

Immunoglobulins were determine by single radial Immunodiffusion method by using ,immuno –kit (Bio Meruex – France) according to (Goldlum &goldman,1977) .[20] .

Results:

Shigella species are a common cause of bacterial diarrhea in developing countries and the diagnosis of shigellosis was considered when the clinical presentation and examination were suggestive , in addition to laboratory diagnosis .

A total of 317 cases suffering from bloody diarrhea enrolled in the study , out of 317 stool samples , 62 bacterial isolates were obtained represent 19.5% from all cases of diarrhea , these isolates are distributed as follows : S*higella dysenteriae* 22(35.4%) , *Shigella sonnei* 19(30.6%) , *Shigella boydii* 13(20.9%) , and *Shigella flexneri* 8(12.9%) table (1) .

Table(1): Number and percentage of shigellosis cases according to *Shigella* species

Shigella Species	No. of cases	Percentage %
Shigella .dysenteriae	22	35.4
Shigella sonnei	19	30.6
Shigella boydii	13	20.9
Shigella flexneri	8	12.9
Total	62	100

Table (2) shows the predominance of shigellosis among below one year age group 32.2% and only nine cases were occur in 2-3 age group represent 14.5% S*higella dysenteriae* was predominance among below one year age group (9 cases) , while only 2 cases were occurred in the 2-3 age group most cases of *Shigella sonnei* were occurred in 2-3 age group . *Shigella boydii* was predominance in below one year age group (3 cases) . most of shigellosis cases in 1-2 age group (4 cases) table(2) .

Table(2) : Relationship between age group of children and *Shigella* species

Shigella species	Total	Age group				
		Below one year	1 ---2	2 --- 3	3 --- 4	4 --- 5
Shigella.dysenteriae	22	9	4	2	3	4
Shigella sonnei	19	5	3	6	2	3
Shigella boydii	13	3	4	0	3	3
Shigella flexneri	8	3	2	1	2	0
Total	62 (100%)	20 (32.2%)	13 (20.9%)	9 (14.5%)	10 (16.1%)	10 (16.1%)

Table (3) and fig. (1) shows the concentration of total protein which showed normal level of total protein in all age groups , while albumin was decreased , alpha 1 – globulin concentration in the serum of children with shigellosis was increased while alpha 2 –globulin was increase slightly with advancing age , beta – globulin concentration in serum of children patients was normal , gamma globulin concentration was slightly elevated in most age groups when compared with normal value .

Table(3) : Concentration of serum proteins in children with shigellosis according to age Groups

Serum Proteins	Age group (years)				
	Below one year	1 — 2	2 — 3	3 — 4	4 — 5
Total protein	*6.8 **6.7 ***(4.4 – 7.6)	6.3 7.0 (5.6–7 .5)	6.1 6.5 (5.6 – 8)	7.6 7.1 (5.6 – 8)	7.8 6.9 (5.6 – 8)
Albumin	2.5 3.7 (3.2 – 5.7)	2.9 4.1 (3.8–5.4)	2.9 3.9 (3.8–5.4)	3.2 4.2 (3.8–5.4)	3.1 4.6 (3.8–5.4)
α -1 globulin	0.8 0.3 (0.1 – 0.3)	0.6 o.2 (0.1 – 0.3)	0.6 0.2 (0.1 – 0.3)	1.4 0.1 (0.1 – 0.3)	1.7 0.3 (0.1 – 0.3)
α -2 globulin	0.6 0.9 (0.2 – 1.1)	0.7 0.6 (0.4 – 0.8)	0.7 0.8 (0.4 – 0.8)	1.0 0.7 (0.4 – 0.8)	0.9 0.7 (0.4 – 0.8)
β – globulin	0.7 0.7 (0.3 – 1.0)	0.4 0.7 (0.5 – 1.0)	0.5 0.8 (0.5 – 1.0)	0.6 0.7 (0.5 – 1.0)	0.8 0.6 (0.5 – 1.0)
γ – globulin	1.2 0.9 (0.2 – 1.2)	1.3 0.7 (0.4 – 1.3)	1.1 0.8 (0.4 – 1.3)	1.9 0.9 (0.4 – 1.3)	2.1 0.9 (0.4 – 1.3)

*The concentration in milligram/100 ml

** The concentration in milligram/100 ml for control group

*** Normal value (Henry *et al* ,1984), [21].

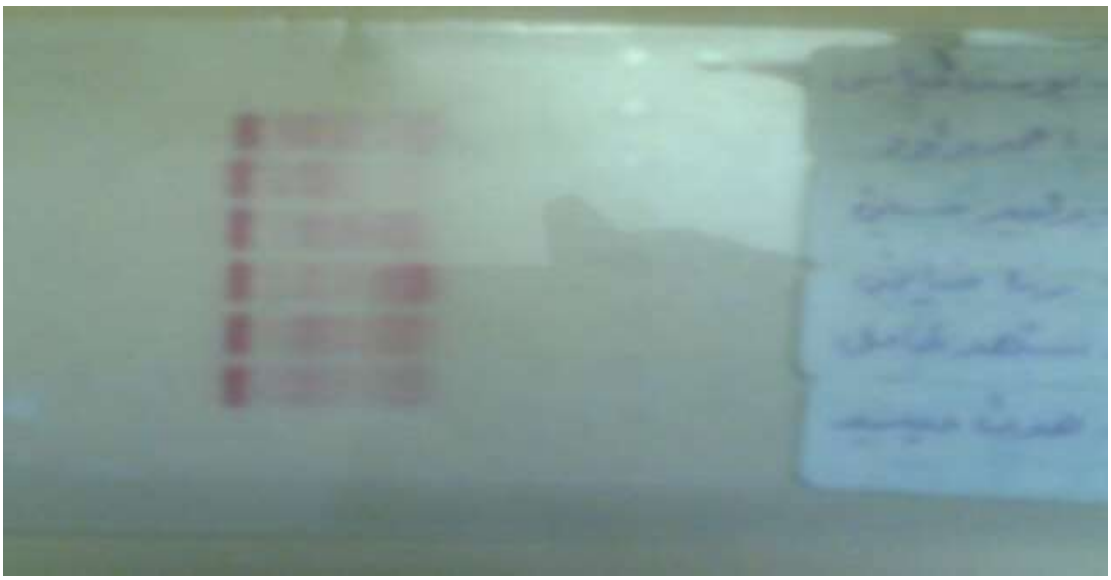

Fig.(1): Bands of Serum proteins of children with Shigellosis

Serum concentration of IgG ,IgM and IgA are measured by radial immunodiffusion using partigens supplied by (Bio Mereux – France) .

Forty – seven children patients 75.8% were found to have high level of lmmunoglobulin concentration while fifteen children patient , 24.2% have low levels of lmmunoglobulin concentration compared to normal value they are classified as follows : 11.2% have IgA deficiency , 5.1% have IgM deficiency and 4.8% have IgG deficiency table [3 , 4 , 5].

Table (4): IgG concentration in serum of children with Shigellosis according to age group

Age group	Total	* Normal conc. **(mg/dl) X±S	Conc. Of IgG (mg/ dl) Control group	Conc. Of IgG (mg/ dl) X± S Patients	"P" Value
Below one year	20	661 ± 219	603±211	787.8± 362.7	P< 0.001
1 ---- 2	13	762± 209	821±228	846.2 ± 392.4	P< 0.001
2 ---- 3	8	929± 228	960±219	1034.4± 387.3	P< 0.001
3 ---- 4	10	929 ± 228	930±231	1125.3±283.7	P< 0.001
4 ---- 5	11	929 ± 228	941±223	1239.8± 272.6	P< 0.001

* Maxwell *et al* ,1976 , [22]. ** dl: decilitre

Table (5) : IgM concentration in serum of children with Shigellosis according to age group

Age group	Total	Normal conc. (mg/dl) X ±S	Conc. Of IgM (mg/ dl) X ± S Control group	Conc. Of IgM (mg/ dl) X ± S Patients	"P" Value
Below one year	20	43±17	48±11	54.7± 17.7	P< 0.001
1 ------ 2	13	58±23	59±21	58.4± 39.2	P< 0.001
2 ------ 3	8	56±18	57±15	134.4 ± 387.3	P< 0.001
3 ------ 4	10	56±18	58±12	132.5±28.7	P< 0.001
4 ------ 5	11	56±18	56±17	139. 6± 72.2	P< 0.001

* Maxwell *et al* ,1976

Table (6): IgA concentration in serum of children with Shigellosis according to age group

Age group	Total	Normal conc. (mg/dl) X±S	Conc. Of IgA (mg/ dl) X ± S Control group	Conc. Of IgA (mg/ dl) X ± S Patients	"P" Value
Below one year	20	37±18	38±12	18.6± 16.2	P< 0.001
1 ---- 2	13	50+24	52±18	43.3±13.2	P< 0.001
2 ---- 3	8	50±24	50±21	104.6 ± 37.8	P< 0.001
3 ---- 4	10	93±27	95±19	112.3±28.7	P< 0.001
4 ---- 5	11	93±27	93±22	129.3± 77.5	P< 0.001

* Maxwell *et al* ,1976

Discussion :

Four species of *shigella* are pathogenic for man . S*higella dysenteriae* causes both epidemic and endemic shigellosis , and this bacterium associated with the most severe disease and the highest case fatality rates [10] . *Shigella sonnei* and *Shigella boydii* usually cause relatively mild illness in which diarrhea may be watery and bloody [5] .*Shigella flexneri* . is the chief cause of endemic shigellosis in developing contries [10 , 23] . The study showed that the percentage of shigllosis among children 19.5% from all cases of diarrhea . these result go with [24] . in this study we found S*higella dysenteriae* was predominance cause of shigellosis ,while in Egypts Nile Delta the *Shigella flexneri* being the most common isolated from children less than three years [25] .

During the past 30 years , pandemics of S*higella dysenteriae* have spread across central America , Bangladesh , South Asia and Central and East Africa [26 , 27 , 28] less frequency of *Shigella* species in this study was *Shigella flexneri* while *Shigella flexineri* is the main serogroup found in developing contries median 60% [29] in our study *Shigella sonnei* was the second cause of shigellosis this result go with other report[30] . followed by *Shigella boydii* and *Shigella flexneri* :[30]. Most of shigellosis cases were found in below one year age group a similar result had been found in study conducted by [30 , 31] . among children the risks of death from shigellosis is greatest in infant and those who are severely malnourished [9].

Our results showed the concentration of the total protein in serum of children with shigellosis, generally was in the normal level in all age groups ,while its increase slightly with advancing age but the concentration of the total protein was still in the normal range in each age group ,this result go with [32]. Albumin concentration was found decreased when compared with normal levels ,similar

result was found by [33].Decreased serum albumin may result from liver and kidney diseases which allows albumin to escape into urine ,while an increase in the percentage of albumin can indicate a severe loss of water from the bloodstream[34]. Albumin concentration was decreased in children with bacterial meningitis whom under five years old [32] . The second component of serum protein is globulins,the globulins are roughly divided into alpha ,beta ,and gamma globulins, alpha globulins include alpha 1 globulin and alpha 2 globulin [34]. Alpha 1 globulin was increased in all age groups , similar result was observed by[32] , and this may be due to complement concentration which is occur according to the infection, while alpha 2 globulin concentration was normal range in most age group this result go with [32]. Beta globulin concentration was normal ,an increase in the percentage of beta globulin may caused by liver disease, while the decrease of beta globulin level may caused by kidney disease or a problems with blood clotting process. Gamma globulin was increased in all age groups this result was expectant due to immune response according to bacterial invasion ,the an increase in the level of gamma globulin may be caused by a chronic infection [34] , while a decrease in the percentage of gamma globulin may be caused by kidney disease or a problems of the immune system. Serum concentration of IgG, IgM, and IgA were measured by radial immundiffusion and our result showed that 75.8% of children patients with shigellosis have high level of immunoglobulins ,while 24.2% have low level of immunoglobulins,this immune deficiency may due to problems in the immunity system this result go with [32] .

Some diseases cause hypogammaglobulinemia (acondition characterized by low level of gamma globulin antibodies such as IgG which predominant during infection and IgA which is distribution of serum antibodies against different common bacterial , viral , and food antigens[15 , 16 , 17].

The IgG , IgM and IgA deficiency lead to response to infection . The changes in IgM levels in patients were significantwhen compared with control group and normal range, so IgG and IgA, and this may be due to invasion by gram negative bacteria or due to poor diet (malnutrition) and gastrointestinal. Which caused by Shigella species. [20] . Shigellosis induced a rise of anti-LPS of all three Ig , the increase in IgG is the highest and has the longest duration (35 36). The Ig response is related to the severity of symptoms, and anti-LPS levels of the three Ig types decline to those in acute-phase sera in less than 1 year (36, 37). . The higher and longer-lasting levels of IgG suggest that our conjugates will induce a more complete and long-lasting immunity than shigellosis.[36]

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تقييم الكلوبيولينات المناعية في مصل الاطفال المصابين بداء الشيغلات

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الخلاصة

اجريت هذه الدراسة في مدينة الناصرية للفترة من الاول من كانون الثاني ٢٠٠٦الى نهاية شهر ايلول من نفس العام على الاطفال المصابين بالاسهال و الذين تراوحت اعمارهم من يوم واحد الى خمس سنوات. تم فحص ٣١٧ عينة غائط ممن كانوا يعانون من الاسهال الدموي ووجد من خلال هذه الدراسة ان نسبة الاطفال المصابين بداء الشيغلا هو ١٩,٥ % وكان عدد العزلات كما ياتي : ٢٢ عزلة لبكتريا*Shigella dysenteriae* ، ١٩ عزلة لبكتريا *Shigella sonnei* ، ١٣ عزلة لبكتريا *Shigella boydii* ، و ٨ عزلات لبكتريا *Shigella flexneri* . اوضحت الدراسة ان نسبة الاصابة في الفئة العمرية (اقل من سنة) كانت اعلى مماهو علية في الفئات العمرية الاخرى. ووجد من خلال هذه الدراسة ايضا ان هنالك ارتفاع في تركيز بعض بروتينات مصل الدم مثل alpha 1 – globulin و alpha 2 –globulin , gamma globulin ، بينما كان albumin دون مستوياتة الطبيعية وبقي تركيز البروتين الكلي عند مستواه الطبيعي وفي كل الفئات العمرية . ووجد ايضا ان هنالك عوز مناعي عند الاطفال المصابين بداء الشيكلا اذ وجد ان هنالك عوز بنسبة ١١,٢% فيما يخص IgA ، اما IgM وIgG فقد كانت نسبة العوز فيهما ٥,١% و ٤,٨% على التوالي .